



Complexation of arsenite with dissolved organic matter: Conditional distribution coefficients and apparent stability constants

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ABSTRACT

The complexation of arsenic (As) with dissolved organic matter (DOM), although playing an important role in regulating As mobility and transformation, is poorly characterized, as evidenced by scarce reporting of fundamental parameters of As–DOM complexes. The complexation of arsenite (As^{III}) with Aldrich humic acid (HA) at different pHs was characterized using a recently developed analytical technique to measure both free and DOM-bound As. Conditional distribution coefficient (K_D), describing capacity of DOM in binding As^{III} from the mass perspective, and apparent stability constant (K_s), describing stability of resulting As^{III}–DOM complexes, were calculated to characterize As^{III}–DOM complexation. Log K_D of As^{III} ranged from 3.7 to 2.2 (decreasing with increase of As/DOM ratio) at pH 5.2, from 3.6 to 2.6 at pH 7, and from 4.3 to 3.2 at pH = 9.3, respectively. Two-site ligand binding models can capture the heterogeneity of binding sites and be used to calculate K_s by classifying the binding sites into strong (S1) and weak (S2) groups. Log K_s for S1 sites are 7.0, 6.5, and 5.9 for pH 5.2, 7, and 9.3, respectively, which are approximately 1–2 orders of magnitude higher than for weak S2 sites. The results suggest that As^{III} complexation with DOM increases with pH, as evidenced by significant spikes in concentrations of DOM-bound As^{III} and in K_D values at pH 9.3. In contrary to K_D , log K_s decreased with pH, in particular for S1 sites, probably due to the presence of negatively charged H₂AsO₃⁻ and the involvement of metal-bridged As^{III}–DOM complexation at pH 9.3.

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1. Introduction

The occurrence of high levels (e.g., >50 μg L⁻¹) of arsenic (As), a notoriously toxic element, in groundwater has posed a human health risk to millions of people worldwide (National Research Council, 1999; Matschullat, 2000; Mandal and Suzuki, 2002; Smedley and Kinniburgh, 2002; Anawar et al., 2003; Tseng, 2005). The release of As from aquifer materials, soils, and sediments into groundwater is controlled by a number of factors (Stollenwerk, 2003; Swartz et al., 2004; Harvey et al., 2005; O'Shea et al., 2006; Tufano and Fendorf, 2006). Among them, dissolved organic matter (DOM) plays an important role because it is ubiquitous in aquatic environments and can interact strongly with As species (Kalbitz and Wennrich, 1998; Harvey et al., 2002; McArthur et al., 2004; Bauer and Blodau, 2006; Wang and Mulligan, 2006). The formation of As–DOM complexes is important in regulating the transport, transformation, and bioavailability of As in the environment (Grafe et al., 2001; Wang and Mulligan, 2006). Through forming complexes, DOM may retain more As in the aqueous phase and thus increase its mobility (Grafe et al., 2001, 2002; Redman et al., 2002;

Cano-Aguilera et al., 2005). The formation of As–DOM complexes, however, could reduce the bioavailability of As and influence the transformation and toxicological effect of As (Wang and Mulligan, 2006).

Only a few studies have documented the formation of As–DOM complexes, although this complexation may critically affect the mobility and toxicity of As species (Redman et al., 2002; Lin et al., 2004; Warwick et al., 2005; Buschmann et al., 2006; Chen et al., 2006; Ritter et al., 2006; Ko et al., 2007). Redman et al. (2002) observed that four of six DOM samples tested were able to form aqueous complexes with arsenate (As^V) and arsenite (As^{III}), with the extent of complexation varying from 20% to 70%. Ritter et al. (2006) reported the association between DOM and As^V, particularly in the presence of Fe^{III}, demonstrating the metal-bridged complexation between As^V, Fe^{III}, and DOM. Buschmann et al. (2006) estimated that 26% of As^{III} and 62% of As^V spiked into Aldrich humic acid solution were bound to DOM under environmentally relevant conditions. Chen et al. (2006) observed that the majority of As present in soil extracts was associated with DOM of molecular weight between 500 and 3500 Da derived from peat amendment.

Despite the aforementioned efforts in studying the interactions between As and DOM, the complexation of As with DOM remains

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unclear in many aspects. For example, many fundamental parameters in a quantitative description of DOM interactions with As, including the conditional distribution coefficients (K_D) and the apparent stability constant (K_s) of the resulting As–DOM (including As–metal–DOM) complexes, are scarce in the literature. Buschmann et al. (2006) determined K_D of 540–3200 L kg⁻¹ for As^{III} and 2000–15 000 L kg⁻¹ for As^V between DOM and water, which are comparable to those reported in a previous study (Thanabalasingam and Pickering, 1986). The K_s values for As–DOM complexes were found in only one study where the average logarithm K_s was determined to be 1.58 for As^{III} and 1.97 for As^V, respectively (Warwick et al., 2005).

The objective of this study was to characterize As^{III} complexation with DOM, derived from Aldrich humic acid (HA), by determining two fundamental parameters of the As^{III}–DOM complexes (K_D and K_s). The present study was focused on As^{III}, one of two predominant As species (As^{III} and As^V) in aquatic environments with higher toxicity. By determining directly both free and DOM-bound As^{III}, which differs from previous studies (Lin et al., 2004; Warwick et al., 2005) where DOM-bound As was often determined by calculating the difference between total and free As, Scatchard plot (Stevenson, 1994) and nonlinear regression were employed to calculate K_s .

2. Methods and materials

2.1. Materials

All chemicals used were of analytical or trace metal grade. All glassware was soaked in 10% HNO₃ overnight and then thoroughly rinsed with deionized water (DIW, 18 MΩ-cm) prior to use. As^{III} standard solution was prepared by dissolving appropriate amounts of sodium meta-arsenite (98%, Sigma–Aldrich, St. Louis, MO, USA) in DIW. Standards for metal analysis were of ICP-MS grade (1000 mg L⁻¹ in 5% HNO₃, GFS Chemicals, Columbus, OH, USA). Aldrich humic acid (HA, sodium salt) was purchased from Sigma–Aldrich. Stock solutions of HA were prepared by dissolving 0.50 g of HA in 100 mL of potassium hydroxide (0.1 M). Sodium nitrate, sodium azide, HCl, and NaOH were purchased from Fisher Scientific (Fairlawn, NJ, USA).

2.2. Analytical methods

The analysis of free and DOM-bound As^{III} was conducted by using a novel technique recently developed in our laboratory, which consists of size exclusion chromatography (SEC) separation, UV/Vis detection for DOM and inductively coupled plasma mass spectrometry (ICP-MS) detection for As (Liu et al., 2008). High performance liquid chromatography (HPLC, SpectraSYSTEM P4000 with quaternary pump and AS3000 autosampler, Thermo Separation Products, Waltham, MA, USA) equipped with a SEC column (Shodex OHPak SB-802.5 HQ, 30 cm × 8.0 mm × 6.0 μm, Showa Denko America Inc., New York, NY, USA) was used in the separation of As^{III} and As^{III}–DOM complex. A SpectraSYSTEM UV150 detector (Thermo Separation Products, Waltham, MA, USA) was used to monitor the DOM peak at 254 nm, while ICP-MS (HP4500 PLUS, Agilent Technologies, Palo Alto, CA, USA) was connected to the SEC system to detect As. Sodium nitrate (0.02 M) was used as the mobile phase with a flow rate of 1 mL min⁻¹. Dissolved organic carbon (DOC) was measured using a Shimadzu TOC 5000 analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). Concentrations of selected elements (Al, Mg, Mn, and Fe) in HA solutions were analyzed by ICP-MS (Mg and Mn) or by atomic absorption spectrometer (AAAnalyst 600, PerkinElmer, Shelton, CT; Fe and Al).

pH was measured on an AR15 pH meter (Fisher Scientific, Fairlawn, NJ, USA).

2.3. Complexation experiments

When used in complexation experiments, 5 mL of HA stock solution was diluted in 495 mL DIW and filtered through 0.45 μm PTFE membrane filters (Corning Inc., Corning, NY, USA). After dilution, the experimental HA solution contained 14 mg L⁻¹ of DOC, and 19, 20, 32, and 72 μg L⁻¹ of Al, Mg, Mn, and Fe, respectively. The complexation of As^{III} with DOM was conducted by adding different concentrations (0–5000 μg L⁻¹ for pH 5.2 and 9.3 while 0–1000 μg L⁻¹ for pH 7.0 due to accidental loss of 5000 μg L⁻¹ treatment at pH 7.0) of As^{III} into 10 mL of HA solutions in 15-mL HDPE centrifuge tubes. Sodium azide (0.02%) was added to the centrifuge tubes to minimize microbial oxidation of As^{III}. The centrifuge tubes were shaken continuously at 300 rpm for 24 h. The mixtures were then centrifuged at 1700g for 10 min and the supernatants were decanted and filtrated through 0.45 μm PTFE membrane filters. After filtration the solutions were immediately analyzed for free and DOM-bound As^{III}. The experiments were conducted at three different pH levels (5.2, 7.0, and 9.3) in order to investigate the effects of pH on As^{III} complexation with DOM.

2.4. Quality control

Strict quality control procedures were followed throughout the experiments, sample analysis, and data analysis. Control experiments suggested that the adsorption of As^{III} by HDPE vials or PTFE membranes used in the experiments were negligible (recoveries for total As determination within 95–105%). Preliminary experiments showed that shaking for 24 h was sufficient for As^{III}–DOM complexation reaching equilibrium. Conversion of As^{III} to As^V during the course of the experiment was not observed. The pH differences between the beginning and end of experiments did not exceed ±0.1. All experiments were conducted in duplicate. The relative percent difference between duplicates was always better than 15%, and the average was reported throughout unless otherwise stated. The 95% confidence intervals were provided for statistical data analysis, if applicable. Additional information on quality control can be found in Supporting material.

2.5. Data analysis

The conditional distribution coefficients of As^{III} binding to DOM (K_D , L kg⁻¹) were calculated as follows (Buschmann et al., 2006):

$$K_D = \frac{[As]_b}{[As]_f \times [DOC]} \quad (1)$$

where $[As]_f$ and $[As]_b$ are free and DOM-bound As^{III} concentrations in μg L⁻¹; and $[DOC]$ is the concentration of DOC in solution in kg L⁻¹.

The stability of As^{III}–DOM complex in solution was estimated using an apparent stability constant (K_s , M⁻¹) (Warwick et al., 2005)

$$K_s = \frac{C_b^{As}}{C_f^{As} \times C_{DOM}} \quad (2)$$

where C_f^{As} and C_b^{As} are free and bound As^{III} concentrations in M; and C_{DOM} is the molar concentration of effective ligands contained in DOM in M. Due to the lack of accurate molecular structure for DOM and the unclear mechanism of DOM complexation with As, C_{DOM} is usually unavailable, which could be part of reason why K_s was scarcely reported. The Scatchard plot (also called Rosenthal

plot) and ligand binding model were used here to determine K_s . The Scatchard plot, widely used in protein chemistry, has been applied to the determination of metal humate stability constants (Stevenson, 1994; Yates and Von Wandruszka, 1999; Guardado et al., 2005). Based on the Scatchard equation, C_b^{As}/C_f^{As} was plotted against C_b^{As} to check the homogeneity of binding sites present in DOM

$$\frac{C_b^{As}}{C_f^{As}} = -K_s \times C_b^{As} + K_s \times B_{\max} \quad (3)$$

where B_{\max} is the maximum binding capability of DOM toward As^{III} in mol L^{-1} . Ligand binding model was then selected to fit the experimental data to calculate K_s and B_{\max} . After K_s and B_{\max} were determined through this nonlinear regression fitting, Scatchard lines for the individual sites were obtained by connecting the B_{\max} and $K_s \times B_{\max}$ values.

The two K parameters (K_D and K_s) calculated here can provide different and complementary information with respect to As complexation with DOM. K_D characterizes the capacity of DOM to bind As from the mass perspective and is useful for estimating the amount of As bound by DOM (Buschmann et al., 2006). K_s is a prime parameter to quantitatively describe interactions between metal and ligand in a stoichiometric way (Riggle and von Wandruszka, 2005). Both K_D and K_s are useful when predicting the fate of As in aquatic environments using a speciation model (Stumm, 1992).

3. Results and discussion

Concentrations of DOM-bound As^{III} , K_D , and K_s were used to characterize As^{III} -HA complexation. The effects of pH As^{III} -HA complexation on these parameters were discussed by comparing these parameters at different pHs.

3.1. Free and DOM-bound As^{III} concentrations

SEC-UV-ICP-MS analyses of As^{III} speciation revealed that only a small fraction of As^{III} was complexed with DOM in the presence of HA (Fig. 1). At pH 5.2, the concentrations of As^{III} complexed by DOM ranged from 0.32 (initial As^{III} = 5.0 $\mu\text{g L}^{-1}$) to 8.2 $\mu\text{g L}^{-1}$ (initial As^{III} = 5000 $\mu\text{g L}^{-1}$), which corresponded to 5.6–0.2% of total As^{III} in solution, respectively. Slightly more As^{III} was complexed by HA at pH 7.0, with the concentrations of DOM-bound As^{III} ranging from 0.54 $\mu\text{g L}^{-1}$ (initial As^{III} concentration = 5.0 $\mu\text{g L}^{-1}$) to 10 $\mu\text{g L}^{-1}$ (initial As^{III} = 1000 $\mu\text{g L}^{-1}$), corresponding to 11–1.1% of total As^{III} . However, at pH 9.3, the concentrations of DOM-complexed As^{III} increased significantly (paired t -test, $p < 0.01$), ranging from 0.85 $\mu\text{g L}^{-1}$ (20% of solution As^{III} , initial As^{III} = 5 $\mu\text{g L}^{-1}$) to 98 $\mu\text{g L}^{-1}$ (2.3% of solution As^{III} , initial As^{III} = 5000 $\mu\text{g L}^{-1}$) (Fig. 1). For all pHs, it is evident that the proportions of DOM-complexed As^{III} to total solution As^{III} decreased with increasing initial As concentrations.

3.2. Conditional distribution coefficients of As^{III} between water and DOM

The logarithm conditional distribution coefficients ($\log K_D$) of As^{III} with DOM ranged from 3.7 to 2.2 at pH = 5.2, from 3.6 to 2.6 at pH = 7.0, and from 4.3 to 3.2 at pH = 9.3, respectively (Fig. 2 and Table 1). These $\log K_D$ values have approximately comparable orders of magnitude with those reported in the literature (2.7–3.5 for HA within pH 4.6–8.4) (Thanabalasingam and Pickering, 1986; Buschmann et al., 2006). K_D decreased rapidly with the increase of initial As^{III} concentrations (Fig. 2), more accurately, with the increase of As/DOC ratios since DOC concentrations were con-

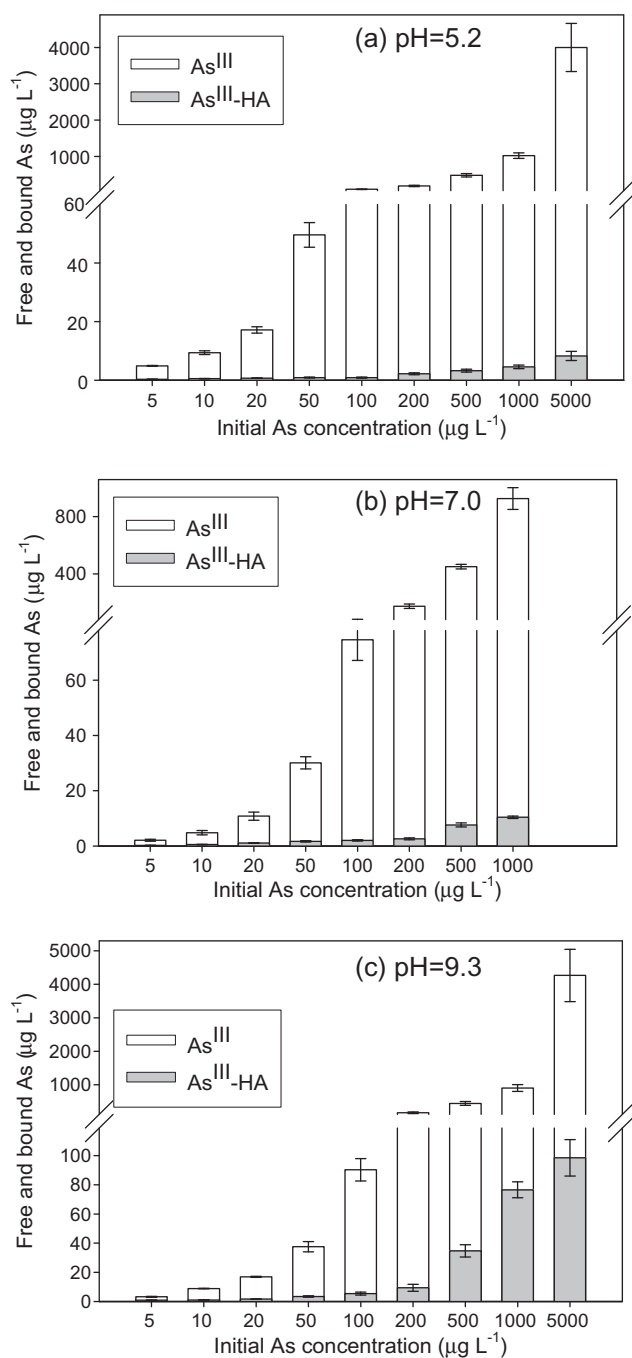


Fig. 1. Concentrations of free arsenite (As^{III}) and DOM-bound arsenite (As^{III} -HA) (y axis) in humic acid (HA) solutions (DOC = 14 mg L^{-1}) at: (a) pH = 5.2, (b) pH = 7.0, and (c) pH = 9.3. The initial concentrations of As^{III} spiked into the HA solutions were denoted on x axis.

stant for each experiment. The sharp decrease in K_D values occurred when the initial concentrations of As^{III} were low (5–100 $\mu\text{g L}^{-1}$, corresponding free As less than 1.2 μM in Fig. 2). At high initial As^{III} concentrations (>100 $\mu\text{g L}^{-1}$), there is little change in K_D values as a function of free As in solution, as evidenced by almost a flat curve for concentrations greater than 2–5 μM in Fig. 2. The relationship between K_D and free As^{III} and DOC could be described by an equation as shown in Fig. 2. This equation is useful for estimating K_D values at given As^{III} and DOC concentrations when using a speciation model to predict As fate in aquatic environments (Buschmann et al., 2006).

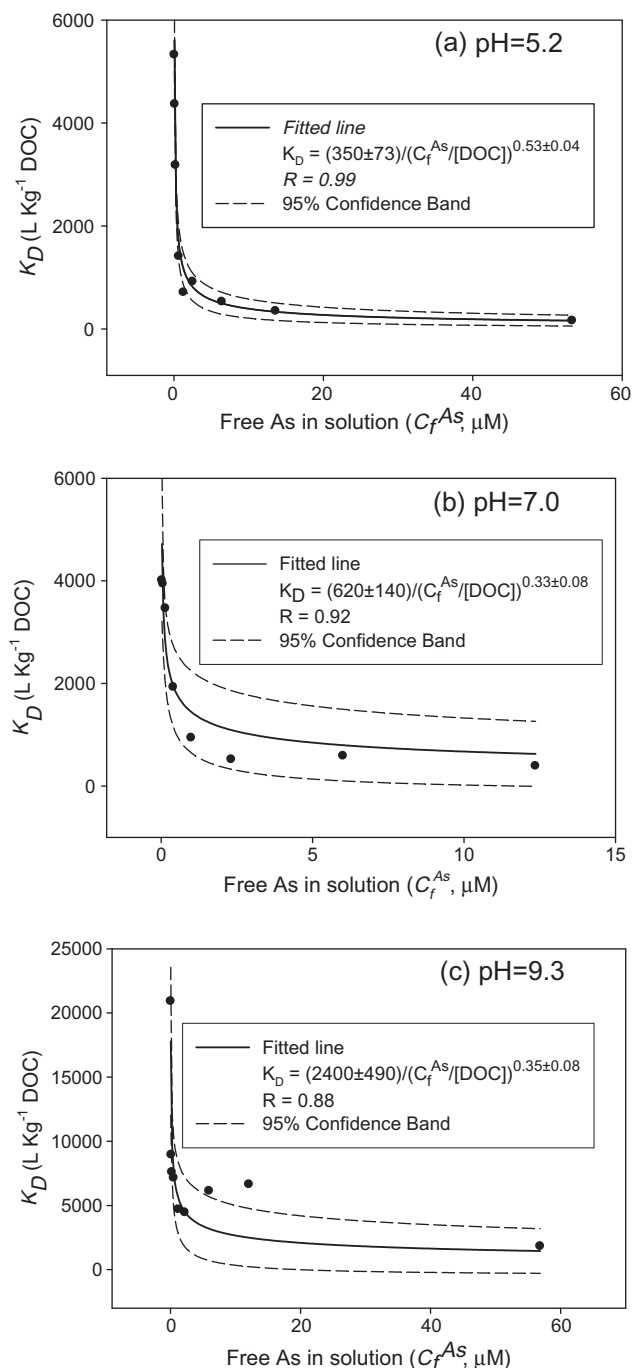


Fig. 2. Conditional distribution coefficients (K_D , y axis) of arsenite (As^{III}) between water and dissolved organic carbon (DOC) as a function of free As^{III} (C_f^{As} , x axis) to DOC concentration ratio in humic acid solutions ($\text{DOC} = 14 \text{ mg L}^{-1}$) at: (a) pH = 5.2, (b) pH = 7.0, and (c) pH = 9.3. Data points represent experimental results. Solid lines are fitted lines with nonlinear regression. Dashed lines show 95% confidence bands for the regression.

Table 1

Conditional distribution coefficients (K_D , L kg^{-1}), apparent stability constants (K_s , M^{-1}), and maximum binding capabilities (B_{max} , μM) of arsenite (As^{III}) to DOM derived from humic acid (HA). DOC concentration was 14 mg L^{-1} for HA solution. Nonlinear regression was performed by fitting experimental data with two-site model of ligand binding. S1 and S2 represent strong and weak binding sites, respectively. The numbers in parentheses are the 95% confidence intervals.

pH	Log K_D	Log K_s		B_{max}		Coefficient of determination (r^2) for nonlinear regression
		S1	S2	S1	S2	
pH = 5.2	3.7–2.2	7.0 (6.9–7.2)	4.6 (4.5–5.0)	0.010 (0.0012–0.019)	0.14 (0.12–0.17)	0.99
pH = 7.0	3.6–2.6	6.5 (6.2–7.1)	4.8 (4.7–4.9)	0.011 (0.0080–0.014)	0.31 (0.28–0.34)	0.97
pH = 9.3	4.3–3.2	5.9 (5.8–6.2)	4.9 (4.7–5.3)	0.21 (0.050–0.38)	1.0 (0.88–1.2)	0.96

3.3. Apparent stability constants of As^{III} -DOM complexes

Consistent with varying K_D values at different initial As^{III} concentrations and nonlinear relationship between K_D and free As^{III} concentration, the Scatchard plots of $C_b^{\text{As}}/C_f^{\text{As}}$ versus C_b^{As} for As^{III} and DOM interactions deviated from linearity for all pHs (Fig. 3), indicating the heterogeneity in the chemical nature of the binding sites present in the DOM derived from HA. One-site model of ligand binding is unable to capture such heterogeneity among the binding sites of HA toward As. Therefore we fitted the experimental data with multiple-site ligand binding models and found that the results of two-site model fitting corresponded to coefficients of determination of at least 0.96 (Table 1). As can be seen from Fig. 3, two distinct slopes were clearly present, suggesting that at least two classes of binding sites appeared to be involved in the complexation of As^{III} with DOM. One class of sites accounted for complexation occurred at low As^{III} concentrations (low $\text{As}^{\text{III}}/\text{DOC}$ ratios) with higher stability (strong complexation sites, S1 sites), while the other corresponded to high As^{III} (higher $\text{As}^{\text{III}}/\text{DOC}$ ratios) with lower stability (weak complexation sites, S2 sites).

Nonlinear regression with two-site ligand binding model suggested that the log K_s for the strong complexation reactions (S1 sites) were 7.0, 6.5, and 5.9 for pH 5.2, 7.0, and 9.3, respectively (Table 1). For the weak sites (S2 sites), the K_s values were 1–2 orders of magnitude lower, with log $K_s = 4.6, 4.8, \text{ and } 4.9$ for pH 5.2, 7.0, and 9.3, respectively. B_{max} for S1 sites was 0.010, 0.011, and 0.21 μM , whereas the values for S2 sites were 0.14, 0.31, and 1.0 μM at pH 5.2, 7.0, and 9.3, respectively (Table 1). B_{max} for S1 sites is an order of magnitude lower than for S2 sites, indicating that the strong sites are far less than the weak ones in HA. The inflexion point in Fig. 3, where the two models (S1 and S2) intersect, occurred at $C_b \approx 0.01\text{--}0.02 \mu\text{M}$ ($C_b/C_f \approx 0.01\text{--}0.03$) for pH 5.2 and 7.0 while at $C_b \approx 0.2\text{--}0.3 \mu\text{M}$ ($C_b/C_f \approx 0.06\text{--}0.07$) for pH 9.3. Comparing the C_b at the inflexion point to B_{max} for S1 sites, it seems that the region around the inflexion point is influenced by both strong and weak complexation, whereas away from this point either strong or weak complexation dominates.

The previous study reported that the log K_s of As^{III} -Aldrich HA complexes ranged from about 0.5 to 2.5 depending on pH and ionic strength, which was calculated based on an assumption of 1:1 stoichiometry of moles of As per mole of binding sites expressed in terms of the proton exchange capacity (PEC) (Warwick et al., 2005). The differences in experimental conditions may account for the difference in K_s values between that study and the present results. For example, higher As (mg L^{-1}) levels and HA concentration (1500 mg L^{-1}) were used by Warwick et al., in comparison to $\mu\text{g L}^{-1}$ level of As and 14 mg L^{-1} of DOC in this study.

HA is a complex mixture of polyfunctional organic acids in which there are many different types of sites with varying binding energies (Stevenson, 1994). At high As concentration (e.g., $>1 \text{ mg L}^{-1}$), most As would be bound to weak sites since the number of strong binding sites is limited and could be easily saturated by As. Therefore, the calculated K_s of As^{III} -DOM complex would be expectedly low, e.g., log $K_s \approx 0.5\text{--}2.5$ as reported by Warwick et al. (2005), at high As concentrations.

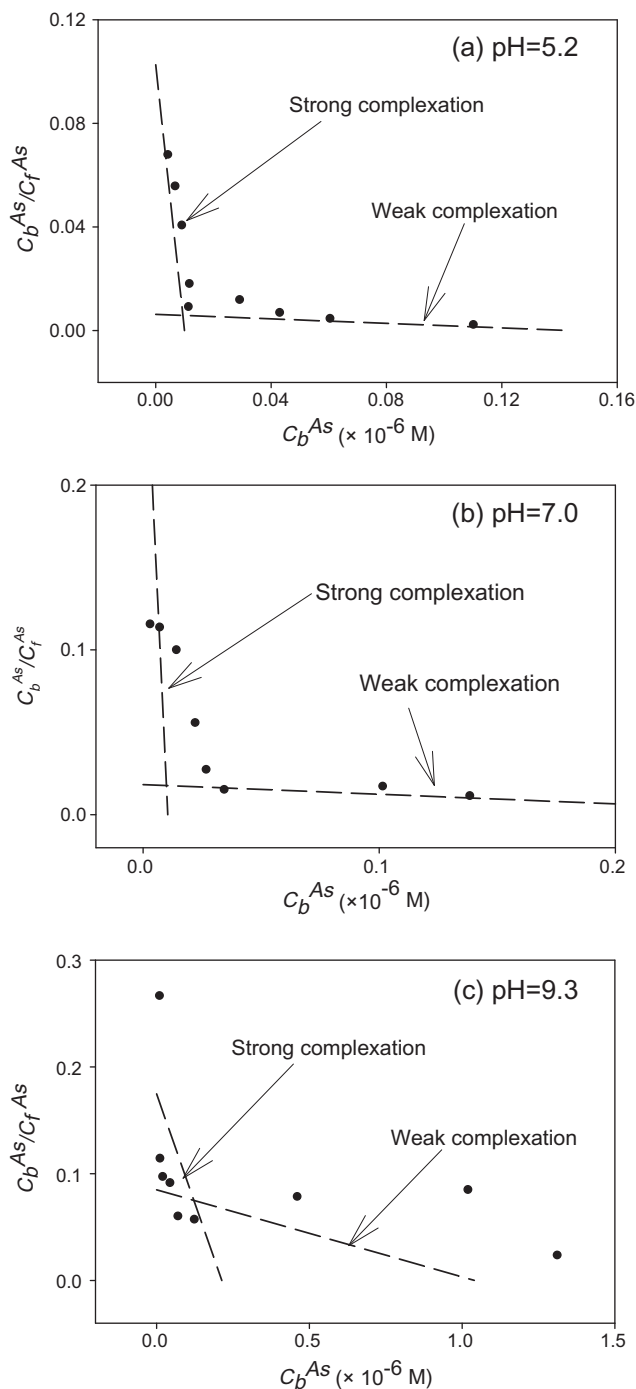


Fig. 3. Scatchard plot (y axis: DOM-bound As^{III} to free As^{III} ratio, C_b^{As}/C_f^{As} ; x axis: DOM-bound As^{III}, C_b^{As}) showing two types of complexation between arsenite (As^{III}) and DOM in humic acid (HA) solutions (DOC = 14 mg L⁻¹) at (a) pH = 5.2, (b) pH = 7.0, and (c) pH = 9.3. Data points represent experimental results. Long dashed lines show Scatchard lines of C_b^{As}/C_f^{As} versus C_b^{As} using B_{max} (maximum binding capability of DOM toward As^{III}, M) and K_s (apparent stability constant of As^{III}–HA, M⁻¹) values determined by nonlinear regression.

The validity of the method used in this study for determining K_s , i.e. combining Scatchard plot to nonlinear regression fitting with ligand binding models, was checked by reprocessing our As^{III}–HA complexation data using the method reported by Warwick et al. (2005). The PEC concentration of HA solution used in our study was estimated based on PEC content reported by Warwick et al. (2005), since both studies used Aldrich humic acid. After recalculation, the log K_s values (5.4–4.5) were obtained for As^{III}–HA com-

plexes, which agree approximately with log K_s for the weak complexation obtained through Scatchard plot and nonlinear regression (4.9–4.6). This agreement suggests that our method for determining K_s values of As^{III}–HA complexes is valid. In addition, our method is capable of determining K_s for different groups of binding sites, accounting for the heterogeneity of binding sites in DOM. However, the two-site model fitting used here cannot fully represent the heterogeneity of binding sites, despite all coefficients of determination being greater than 0.96. For instance, the coefficient of determination at pH 9.3 appears to over-represent the actual linear regression of the weak complexation (Fig. 3c), where the data points do not match the suggested model nearly as well as in the cases of pH 5.2 and 7.0 (Fig. 3a and b). Ligand binding models with more than two sites might be needed to more accurately represent the heterogeneity of binding sites.

3.4. pH Effect on As^{III}–DOM complexation

Comparing the concentrations of As^{III} complexed by DOM at different pH, it was observed that maximum complexation occurred at basic pH (pH = 9.3 in this study) and complexation at both neutral (7.0) and acidic (5.2) pH values decreased by an order of magnitude, with slightly higher complexation at pH = 7.0 than at pH = 5.2. As a result, log K_D values of As^{III} complexation with HA were significantly (paired *t*-test, $p < 0.01$) higher at pH 9.3 than at pH 7.0 and 5.2. Ligand binding models also revealed higher B_{max} values at pH 9.3, indicating DOM could bind more As^{III} at higher pH than at lower pH. These results are not in agreement with a previous study (Buschmann et al., 2006), where maximum As^{III}–Aldrich HA binding was observed around pH 7. The discrepancy could be related to different pH ranges studied. The pH range in our study was from 5.2 to 9.3 whereas, the highest pH tested in that study was 8.4 (from 4.6 to 8.4). pK_a values of arsenious acid (H₃AsO₃) are as follows: $pK_1 = 9.22$, $pK_2 = 12.13$, and $pK_3 = 13.4$ (Cullen and Reimer, 1989). In the pH range of 4.6–8.4, As^{III} exists predominantly as a single species of neutral hydroxo complex, As(OH)₃, and thus similar As^{III}–DOM binding mechanisms could be involved for all pHs. In this case, maximum binding at pH 7 is expected since H⁺ competes for humic functional groups at low pH values and OH⁻ competes for the As^{III} center at high pH values (Buschmann et al., 2006). However, at pH 9.3, as is the case in our study, As^{III} is present in two different forms, a neutral (As(OH)₃) and a negatively charged species (H₂AsO₃⁻) (Cullen and Reimer, 1989), with the latter being higher in concentration (by 20% based on theoretical calculation). Different mechanisms would be expected to be involved in As^{III}–DOM binding at pH 9.3 than at lower pH values, since As^{III} exists in different species. Although the issue of what mechanisms were involved at higher pH values (> pK_a) is unresolved, it is possible that more As^{III} was complexed by DOM due to the alteration of As^{III}–DOM binding mechanisms, as we observed in this study.

Contrary to log K_D , the log K_s values decreased with increased pH for S1 sites, from 7.0 to 5.9, as the pH was raised from 5.2 to 9.3 (Table 1). This is not unexpected given that more As^{III} is complexed by DOM at higher pH and thus more weak binding sites would be occupied in addition to the limited number of strong sites. HA is a complex material possessing carboxylic, amino, nitroso, sulfhydryl, hydroxyl, and phenolic moieties (Aiken et al., 1985; Macalady and Ranville, 1998). These functional groups could act as binding sites for As^{III}–DOM complexation, but with different affinity. It should be noted that although a two-site model was used in this study to loosely classify these binding sites into two groups (strong and weak sites) for the purpose of K_s calculation, each of these two groups still includes multiple types of sites. It is likely that stronger sites would be occupied first when As^{III} is bound by DOM. At lower pH, less As is complexed by DOM and thus the As

should be bound to stronger sites, resulting in higher K_s values. At higher pH, more As is complexed by DOM and a large fraction of As would be bound to weaker sites after the stronger sites are occupied, yielding lower K_s values.

Ultimately, the chemical mechanisms dictating As–DOM complexation are related to the strong and weak binding constants described in this study, and are affected by pH. Prevalent mechanisms postulated to interpret the association between DOM and As include ligand exchange between As species and functional groups of DOM, metal-bridged ternary complexation, and hydrophobic interaction (Redman et al., 2002; Lin et al., 2004; Buschmann et al., 2006; Ritter et al., 2006). The functional groups present in HA, such as phenolic, carboxylic, amino, and sulfhydryl moieties, may bind As by forming negatively charged adducts (Buschmann et al., 2006). The direct association of As with these functional moieties may exhibit varying strength and thus represent different binding mechanisms. Furthermore, these functional groups in HA may bind As via a cation (e.g., Fe) bridge binding mechanism by forming DOM–cation–As complexes (Redman et al., 2002; Buschmann et al., 2006; Ritter et al., 2006). At this point, it is unclear what exact mechanisms are involved in the complexation of As^{III} with DOM for the strong and weak sites at different pH values tested in this study. However, As^{III}–metal–DOM binding is probably one form of As–HA association, in particular at pH 9.3. The HA solutions used in this study did have inherent metal contents (e.g., 72 $\mu\text{g L}^{-1}$ for Fe, see Section 2.1). These metals could act as cation bridges and be responsible for the increased As complexation at pH 9.3 where As^{III} exists mainly as the negatively charged form. Like complexation of other anions with DOM through metal bridge (Stevenson, 1994; Riggle and von Wandruszka, 2005), metal-bridged As^{III}–DOM binding constitutes one type of weak complexation.

4. Conclusions

The complexation of As^{III} with DOM derived from Aldrich humic acid was investigated by determining both free and DOM-bound As using a SEC-UV/Vis-ICP-MS analytical technique. In addition to K_D , K_s was calculated by using Scatchard plot and nonlinear regression of multiple-site ligand binding models to characterize As^{III}–DOM complexation. The fractions of As^{III} complexed by DOM, which peaked at 20% for an initial As^{III} concentration of 5.0 $\mu\text{g L}^{-1}$ at pH 9.3, decreased rapidly with the increase in As/DOC ratios for initial As^{III} from 5.0 to 100 $\mu\text{g L}^{-1}$. Correspondingly, $\log K_D$ of As^{III} with HA decreased sharply with the increase of As/DOC ratios. The Scatchard plots of C_b^{As}/C_f^{As} versus C_b^{As} deviated from linearity for all pHs, clearly indicating the heterogeneity in the chemical nature of the binding sites present in the DOM derived from HA. Two-site ligand binding models were then used to calculate K_s of As^{III}–DOM complexes by grouping the heterogeneous binding sites into strong (S1) and weak (S2) sites. The K_s values calculated for S1 sites were higher than for S2 sites by one to two orders of magnitude. However S1 sites were limited in numbers, as evidenced by lower B_{max} values, which were 5–30 times smaller than those for S2 sites. The results obtained for the three pH levels tested (5.2, 7.0 and 9.3) indicate that As^{III} complexation with DOM increases with pH (particularly at pH 9.3), whereas the $\log K_s$ values decreased for S1 sites, and remained stable for S2 sites. These variations at the higher pH are attributed to alterations in As^{III} speciation and in As^{III}–DOM complexation mechanisms.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2010.08.002.

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